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37 C.F.R. 1.8

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5/14/03
Date

Gina N. Shishima

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Conrad

Serial No.: 09/854,412

Filed: May 11, 2001

For: HIGH EFFICIENCY mRNA ISOLATION METHODS AND COMPOSITIONS

Group Art Unit: 1636

Examiner: Katcheves, Konstantina

Atty. Dkt. No.: AMBI:073US

DECLARATION OF RICHARD C. CONRAD, PH.D, UNDER 37 C.F.R. §1.132

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Amended 7/23/03 JC

I, Richard C. Conrad, Ph.D., declare the following:

1. I am an inventor of the above-referenced patent application. I am a Senior Scientist at Ambion, Inc. and have worked there for three years (since March, 2000). I have a Ph.D. in Molecular Biology, which I received in 1987 from the University of Wisconsin at Madison. I was a postdoctoral fellow at Indiana University for nine years and at Eli Lilly and Company for two and a half years, as well as a Facility Manager at Indiana University for two years. I have worked in the field of molecular biology, including

nucleic isolation techniques for approximately twenty-five years. My *curriculum vitae* is attached as Exhibit 1.

2. I understand that the claims in this application have been rejected as not novel or obvious over U.S. Patent No. 5,759,777 issued in the name of Kearney *et al.* ("Kearney patent").

3. I have reviewed the Kearney patent and believe it does disclose or teach my invention.

4. My invention is based on my discovery that some problems with mRNA isolation stems from rRNA carryover that is based not on rRNA interactions with the targeting molecule, such as oligo-dT, but on rRNA interactions with mRNA. *See* specification at page 4, lines 25-28; Examples 1 and 2.

5. The use of TEAC and TMAC minimizes differences in bond strength between A:T and G:C basepairs, as G:C basepairing is known to be stronger than A:T basepairing. Isolation of mRNA based on A:T basepairing is affected in the presence of TEAC or TMAC. Basepairing between the poly(A) stretch at the 3' ends of all non-histone eukaryotic mRNAs and a poly(T) or poly(U) nucleic acid can be positively exploited at the expense of the mixed G:C and A:T basepairing between mRNA and rRNA to reduce the carryover of rRNA. *See* specification at page 4, line 28 to page 5, line 7. Furthermore, I believe the TEAC and TMAC reduce basepairing between the rRNA and mRNA, as well as rRNA and a poly(T) or poly(U) nucleic acid that might be employed to hybridize with the mRNA.

6. Based on my knowledge of the field, I believe that if one did not know or appreciate that rRNA carryover as a contaminant in a mRNA sample can be attributed to hybridization between rRNA and mRNA or between rRNA and a poly(T) or poly(U) nucleic acid, then

that person would not consider the use of TEAC or TMAC in an mRNA isolation procedure.

7. I hereby declare that all statements made of my own knowledge are true and all statements made on information are believed to be true and further that the statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment or both under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

5/8/03
Date

Richard C. Conrad, Ph.D.

Curriculum vitae:

Richard C. Conrad

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Austin, Texas 78749
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Research Background:

SENIOR SCIENTIST *March, 2000 to present*

Research and Development, Ambion, Inc.

-Research and development relating to creation and marketing of molecular biological kits. Primary focus is on kits for the isolation, synthesis, and analysis of RNA.

-Personal projects:

Devised new, more efficient PolyA+ RNA isolation kit (the 'Poly(A) Purist' line)
Aided in the production of higher-quality RNA preparations (polyA+ and total)
Devised kit for the transcription and purification of dsRNA for RNAi experiments
Worked on stabilization of RNA from biological samples

POSTDOCTORAL RESEARCH FELLOW *Sept, 1997 to March, 2000*

Discovery Chemistry Research, Eli Lilly and Company

-Biochemical studies on coagulation/thrombosis in humans. Work centered on the reaction of factor Xa, the penultimate step in fibrin clot formation. Also performed gene expression analysis relating to events associated with thrombosis.

MANAGER, *Jan, 1996 to Aug, 1997*

Center for Aptamer Research, Indiana University Institute for Molecular and Cellular Biology.

-Development of analytical methods based on aptamers, from their use as diagnostic reagents to their potential as *in vivo* inhibitors of specific enzymes. Also established outside collaborations, and began automation effort for *in vitro* selection/amplification.

POSTDOCTORAL RESEARCH ASSOCIATE *1993 to 1995.*

Laboratory of Dr. Andrew D. Ellington. Department of Chemistry, Indiana University.

-*In vitro* selection/evolution of novel functional nucleic acids (RNA and DNA), specifically binders for proteins (aptamers), and analysis of these molecules. Isolated isoform-specific aptamers for protein kinase C, and demonstrated that these are also enzyme inhibitors.

POSTDOCTORAL FELLOW *1987 to 1993.*

Laboratory of Dr. Thomas Blumenthal. Department of Biology, Indiana University.

-Analysis of snRNPs and the signals involved in pre-mRNA processing, primarily trans-splicing, in *C. elegans* using molecular biological, as well as biochemical and immunological techniques. Extensive work with construction of artificial genes, transformation of worms, and analysis of organismal RNA.

GRADUATE RESEARCH ASSISTANT *1978 to 1986.*

Laboratory of Dr. Gary R. Craven. Department of Molecular Biology, University of Wisconsin.

-Biochemical studies on ribosomes of *E. coli* and *S. cerevisiae* and their proteins, demonstrating specific proteins and domains involved in particular ribosome functions. Extensive work with protein fragmentation, modification, and purification. (Last year under Dr. Richard Burgess, due to death of Dr. Craven.)

Teaching Background:

-Handled two discussion sections a week for undergraduate course in general and molecular genetics. *Fall, 1978 and Spring, 1979 semesters.*

-Organized, wrote instructional book, taught and oversaw a one-week workshop in In Vitro Selection offered to the general research audience. *June, 1996.*

Education:

Ph. D., Molecular Biology
University of Wisconsin, 1987

B.A.s, Biology and Chemistry
Indiana University, 1977.

Awards:

Walther Cancer Institute Fellowship, 1995.
NIH training grant in genetics, 1977-1980.
Bachelor's degree with honors in Chemistry, 1977.

Patents:

Patent application SN 09/854,412 "High Efficiency mRNA Isolation Methods and Compositions", Richard C. Conrad, filed May 11, 2001.

Publications:

Xingwang Fang, Roy C. Willis, Michael A. Siano, Manuel Quinto-Pozos, and Richard C. Conrad. 2003. *Automated high-throughput mRNA selection from eukaryotic total RNA.* **J. Assn. Lab. Automation 8:** 51-54.

Sulay D. Jhaveri, Romy Kirby, Rick Conrad, Emily J. Maglott, Michael Bowser, Robert T. Kennedy, Gary Glick, and Andrew D. Ellington. 2000 *Designed Signaling Aptamers that Transduce Molecular Recognition to Changes in Fluorescence Intensity.* **J.A.C.S. 122:** 2469-2473.

Michael R. Wiley, L.C. Weir, S. Briggs, N.A. Bryan, J. Buben, C. Campbell, N.Y. Chirgadze, Richard C. Conrad, T.J. Craft, J.V. Ficorilli, J.B. Franciskovich, L.L. Froelich, D.S. Gifford-Moore, T. Goodson Jr, D.K. Herron, V.J. Klimkowski, K.D. Kurz, J.A. Kyle, J.J. Masters, A.M. Ratz, G. Milot, R.T. Shuman, T. Smith, G.F. Smith, A.L. Tebbe, and J.M. Tinsley. 2000 *Structure-based design of potent, amidine-derived inhibitors of factor Xa: evaluation of selectivity, anticoagulant activity, and antithrombotic activity.* **J. Med. Chem. 43:** 883-99.

Richard C. Conrad. 1999 *Aptamers: Another Use for Oligonucleotides.* In Ronald A. Leslie, A. Jackie Hunter, and Harold A. Robertson (eds.), **Antisense Technology in the Central Nervous System**, Oxford University Press, pp. 195-217.

Radislav A. Potyrailo, Richard C. Conrad, Andrew D. Ellington, and Gary M. Hieftje. 1998 *Adapting selected nucleic acid ligands (aptamers) to biosensors.* **Anal. Chem. 70:** 3419-25.

Richard C. Conrad, Tonia L. Symensma, and Andrew D. Ellington. 1997 *Natural and unnatural answers to evolutionary questions.* **Proc. Natl. Acad. Sci. U.S.A. 94:** 7126-8.

Richard C. Conrad, F. Maike Brück, Sabine Bell, and Andrew D. Ellington. 1998 *In vitro selection of nucleic acid ligands.* In Christopher W. J. Smith (ed.), **Nucleic Acid-Protein Interactions: A Practical Approach**, Oxford University Press, pp. 285-315.

Richard Conrad and Andrew D. Ellington. 1996. *Detecting immobilized protein kinase C isozymes with RNA aptamers.* **Anal. Biochem. 242:** 261-265.

Richard Conrad, Lori Giver, Yu Tian, and Andrew D. Ellington. 1996. *In vitro selection of nucleic acid aptamers that bind proteins.* **Methods in Enzymology, vol. 267,** 336-367.

Richard C. Conrad, Scott Baskerville, and Andrew D. Ellington. 1995. *In vitro selection methodologies to probe RNA function and structure.* **Molecular Diversity 1:** 69-78.

Andrew D. Ellington and Richard Conrad. 1995. *Aptamers as potential nucleic acid pharmaceuticals.* In: M. R. El-Gewely (ed.), **Biotechnology Annual Review. vol. 1,** Elsevier Publishing, pp. 185-214.

Richard Conrad, Kristi Lea, and Thomas Blumenthal. 1995. *SL1 trans-splicing specified by AU-rich RNA inserted at the 5' end of Caenorhabditis elegans pre-mRNA .* **RNA 1:** 164-170.

Rick Conrad, Lisa Keranen, Alexandra Newton, and Andrew Ellington. 1994. *Isozyme-specific inhibition of protein kinase C by RNA aptamers.* **J. Biol. Chem. 269:** 32051-32054.

Richard Conrad, Ruey-Fen Liou, and Thomas Blumenthal. 1993. *Functional analysis of a C. elegans trans-splice acceptor.* **Nucleic Acids Res. 21:** 913-919.

Richard Conrad, Ruey-Fen Liou, and Thomas Blumenthal. 1993. *Conversion of a trans-spliced C. elegans gene into a conventional gene by introduction of a splice donor site.* **EMBO J. 12**: 1249-1255.

Richard Conrad, Jeffrey Thomas, John Spieth, and Thomas Blumenthal. 1991. *Insertion of part of an intron into the 5' untranslated region of a Caenorhabditis elegans gene converts it into a trans-spliced gene.* **Mol. Cell. Biol. 11**: 1921-1926.

John Spieth, Yhong Hee Shim, Kris Lea, Richard Conrad, and Thomas Blumenthal. 1991. *elt-1, an embryologically expressed Caenorhabditis elegans gene homologous to the GATA transcription factor family.* **Mol. Cell. Biol. 11**: 4651-4659.

Jeffrey D. Thomas, Richard C. Conrad, and Thomas Blumenthal. 1988. *The C. elegans trans-spliced leader RNA is bound to Sm and has a trimethylguanosine cap.* **Cell 54**: 533-539.

Li-Ming Changchien, Richard C. Conrad, and Gary R. Craven. *Isolation of fragments of ribosomal proteins that recognize rRNA.* 1988. **Methods in Enzymology 164**: 258-270.

Michael Cannon, Graham J. Threadgill, Robert Mount, and Richard C. Conrad. 1987. *Identification and analysis of yeast ribosomal proteins by high-performance liquid chromatography.* 1987. **Biochemical Society Transactions 15**: 1040-1041.

Richard C. Conrad and Gary R. Craven. 1987. *A cyanogen bromide fragment of S4 that specifically rebinds 16S RNA.* **Nucleic Acids Res. 15**: 10331-10343.

Graham J. Threadgill, Richard C. Conrad, Michael Cannon, and Gary R. Craven. 1987. *A rapid and preparative method for the separation of yeast ribosomal proteins by using high-performance liquid chromatography.* **Biochem. J. 244**: 523-532.

Li-Ming Changchien, Richard C. Conrad, and Gary R. Craven. 1986. *Chemical and functional characterization of an altered form of ribosomal protein S4 derived from a strain of E. coli defective in auto-regulation of the alpha operon.* **Nucleic Acids Res. 14**: 6929-6944.

Graham J. Threadgill, Richard C. Conrad, Li-Ming Changchien, Michael Cannon, an Gary R. Craven. 1986. *Application of high-performance liquid chromatography to the purification and characterisation of yeast ribosomal protein L3 from trichodermin-resistant yeast mutants.* **Biochem. J. 237**: 421-426.

Me ting Presentations:

"Aptamers: Another Use for Oligonucleotides." Invited speaker for an international symposium titled "Antisense Oligonucleotide Gene Knockout in the Nervous System". March 16-19, 1997. Oxford University, Oxford, England.

"The How, What, Where, and Why of Aptamers." Walther Cancer Center Institute Scientific Retreat. September 23, 1996. Indiana University - Purdue University in Indianapolis.

"New Drug Design. " Walther Cancer Center Institute Scientific Retreat. October 7, 1995. Indiana University - Purdue University in Indianapolis.

"Signals for Cis- and Trans-splicing in *C. elegans*." 1992 Midwest *C. elegans* conference, June 5-7, 1992. Washington University, St. Louis, MO.

"Analysis of Splice Site Definition in *C. elegans*." 1991 meeting on *C. elegans*, June 1-5, 1991. University of Wisconsin, Madison, WI.

"Analysis of Splice Site Definition in *C. elegans*." 1991 meeting on RNA processing, May 15-19, 1991. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

"Interconversions Between Trans- and Cis-splice Sites in *C. elegans*." 1990 Midwest Worm Meeting, June, 1990. Indiana University, Bloomington, IN.

"The *C. elegans* SL RNA is a snRNP Consumed During the Trans-splicing process." 1988 Midwest Worm Meeting, June, 1988. Washington University, St. Louis, MO.